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METHODS OF MASS CULTURING MELON FLIES AND ORIENTAL AND MEDITERRANEAN FRUIT FLIES

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A low-cost, efficient means of mass culturing insects is a prerequisite for proper evaluation and use of such fruit fly control methods as the sterile-fly-release technique. Development and improvement of other control procedures can be greatly facilitated by having a dependable, constant source of all stages of the insect. Large numbers of insects are utilized for extensive screening of fumigants, insecticides, chemosterilants, and lures, for breeding parasites, and for biological studies that involve insect movement and field ecology.

In 1949-50 Finney (1956)[2/] and his associates pioneered in developing practical rearing methods for producing several thousand adults per man-day of the oriental fruit fly (Dacus dorsalis Hendel) and the Mediterranean fruit fly [Ceratitis capitata (Wiedemann)], but they were less successful with the melon fly (D. cucurbitae Coquillett).

1/ We gratefully acknowledge the invaluable assistance of Richard Y. Okamoto, Masami Komura, and Clarence W. Akana, of this Division, in developing the mass-production techniques and that of Thomas T. Kozuma, also of this Division, in constructing the colony cages, cabinets, and mechanical pupal sifter.

2/ The year after the author's name is a key to the reference in Literature Cited at the end of this report.

Personnel at the Fruit Fly Investigations laboratory in Honolulu have modified and improved the original methods of Finney, have developed several new procedures, and now have highly efficient production methods for each of the three species. Since 1951 they have reduced the total production costs, including labor, for each species from more than $300 to less than $62 per million pupae. Attainable production per man-day is now more than 1 million flies. From 1960 to 1964, inclusive, they have produced about 1.4 billion pupae (706 and 100 million oriental and Mediterranean fruit fly pupae, respectively, and 595 million melon fly pupae) from about 2.5 billion eggs to support research in Hawaii and the western Pacific eradication programs (Steiner et al. 1965). Except when their rearing program was interrupted temporarily by failure of temperature-control equipment or by logistical supply problems, they have sustained this high production for more than 4 years.

In this report are described the production methods now used to rear the three fruit flies at the Fruit Fly Investigations laboratory, and some comparative results are included from alternative methods that might be adaptable in other situations.

Regulating Temperature, Relative Humidity, and Light

Workers at the Fruit Fly Investigations laboratory have found that one or more stages of each of the three Hawaiian fruit flies develop most rapidly at about 32° C. and that higher temperatures rapidly become harmful. The gain in development rate from 27° to 32° is about equal to that from 24° to 27°.

Before controlled temperatures were used, the temperatures in this laboratory generally were from 24° to 30° C., but rarely exceeded a range from 20° to 32°. Relative humidity usually ranged from 50 to 90 percent during the day and night in dry weather and up to near saturation in rainy periods. High humidity caused adult diets to deliquesce rapidly and the flies to become entangled and die quickly. Many times the stocks were nearly annihilated. Low temperatures slowed larval development and often allowed molds and yeast to contaminate larval media. Under controlled temperatures such conditions no longer occur.

Because little gain in rate of fly development occurs above 27° C., the formulas for food and larval media have been adjusted so that they are most suitable when kept at this temperature. Since regulation of relative humidity is unimportant in the cabinets containing the larval media, it is not controlled there. However, excessive humidity is critical where adult stocks are held because it adversely affects the dry diets now used. Accordingly, dehumidification equipment is used in the separate adult fly laboratory to hold the relative humidity below 80 percent.

Because of Hawaii's latitude, diurnal periods are never too short for adequate feeding, mating, and oviposition of the flies. However, in order to subdue daylight and to equalize light intensity throughout the cages so that flies do not crowd to one side, outside windows have been covered with a thin layer of white paint. Two rows of 8-foot 80-watt fluorescent lamps are operated from 8 to 4:30 daily on the ceiling above all egg-production cages.

Rearing Procedures

The oriental fruit fly strain presently used in the Fruit Fly Investigations laboratory originated from field-infested fruits collected on Oahu in 1950. This strain has been maintained for 14 years without replenishment and with no detectable loss of vigor. From this strain was segregated a second strain having white instead of yellow markings. This color variant is present in 0.5 to 3 percent of oriental fruit fly field populations in different parts of Hawaii. The Mediterranean fruit fly and melon fly laboratory strains from wild flies collected in the field in 1956 and 1958, respectively, have been continuously produced. Previously, laboratory cultures of these species suffered frequent losses and wild flies had to be continually introduced to maintain these cultures.

Recent collections of wild oriental fruit flies, allowed to reproduce in the laboratory cages, required progressively shorter periods for each successive generation to reach sexual maturity. Their adaptation to these laboratory conditions was comparatively rapid. For example, the preovipositional periods of the parent, F_1, and F_2 flies were four, three, and two times, respectively, as long as the preovipositional period of the laboratory-bred flies (7 days at 27° C.). However, by the 10th generation there were no perceptible differences in length of the preovipositional period, fecundity, and longevity. The early delay appeared to be caused, in part, by greater excitability of the wild flies, which affected their response to the dry diet.

The standard adult diet for all species now consists of a low-cost enzymatic yeast hydrolysate[3/] in coarse powder form and granulated sugar, each offered dry and in separate containers, with plenty of fresh water available. This yeast hydrolysate was substituted for a pellet-type yeast (MRT) hydrolysate[4/] in the melon fly diet in 1958. Also in 1958 the Type M dry diet was found superior to, and substituted for, the then standard Mediterranean fruit fly diet. The latter was a mixture of the MRT hydrolysate, honey, and lanoleic acid, which had been suggested by K. S. Hagen of the University of California at Berkeley. In 1962 the present standard diet was substituted for various combinations of enzymatic soy hydrolysate[5/] and yeast hydrolysate plus sucrose, which had been developed as oriental fruit fly diets since the discovery by Hagen and Finney (1950) that yeast hydrolysate could supply most nutrients essential to the three species.

3/ Standard Brands' Fleischmann's Type M. Mention of a proprietary product or company in this report does not constitute a guarantee or warranty of the product or endorsement of the company by the U.S. Department of Agriculture and does not imply approval by the Department to the exclusion of other products or companies that may also be suitable.

4/ From Marvin R. Thompson Co.

5/ From Nutritional Biochemicals Co.

The yeast hydrolysate (Type M) now used was first found to be highly effective in protein hydrolysate-organic phosphate bait-spray formulas (Steiner et al. 1958). It is less hygroscopic than most protein hydrolysates and its nutritional values as well as physical characteristics are responsible for its performance. These dietary changes greatly reduced the cost of materials and labor when they replaced a liquid diet, but they produced no change in fecundity or fertility of the insects. Hatching rates for the last 4 years have averaged 88 and 86 percent for the Mediterranean and oriental fruit flies, respectively, and 93 percent for the melon fly.

In order to meet weekly production goals of 20 million eggs and 10 or more million melon or oriental fruit flies, a bank of 40 stock cages (fig. 1) is maintained at the laboratory. A fourth of these cages is cleaned and restocked each week to insure a complete turnover of insects at 4-week intervals. Mediterranean fruit fly cages are restocked at 3-week intervals and a third of the flies is replaced each week.

Figure 1.--Egg production cages are stacked four high on a base supported by casters. Each cage is set in a shallow pan, which serves as the floor. The pan is covered with a plastic sheet to facilitate cleaning. A detachable pipe brings water to a V-trough that extends through each cage and drains into gutters on the rear wall of the room.

Cages are constructed in two parts to facilitate sterilization and flushing with steam and hot water. The steam kills all surviving flies before the cage is opened (fig. 2). Steam sterilization helps prevent outbreaks of disease and reinfestation by Drosophila sp. The base of each cage consists of a shallow (1 inch deep) aluminum pan. The remainder of the cage is constructed of 3/8-inch weatherproofed plywood and Monel metal hardware cloth (16 mesh per inch). The cage measures 1 by 2 by 4 feet, has a large door in one end panel, and fits snugly into the pan base. The hardware cloth constitutes the top and upper 7 inches of each side. For holding egg receptacles, five circular openings, each measuring 3-3/16 inches in diameter, are evenly spaced on each side. Each opening has a hinged cover. A polyethylene sheet, 26 by 50 by 0.004 inches, is placed on the bottom of each pan

Figure 2.--Both egg-production and media cabinets are cleaned and sterilized with steam. The steam is first used to kill the old flies and to prevent their escape when the cages are opened.

as a covering before the cage is set in it. Cages are stacked four high on a movable base and separated in the stacks by 2-inch wooden blocks on each upper corner to permit entrance of more air and light between the metal pan and the screen top of the cage below.

A 52-inch wick of cotton dental roll, one-half inch in diameter, is placed in an aluminum V-shaped channel that extends the entire length of each cage (3 inches below the top) and out 2 inches at each end. Both ends of the channel are closed except for a drain hole at one end. Each stack of four cages supports a detachable pipe having four small spigots positioned above the troughs (fig. 1). These are suspended on the front side of the stack of cages. When partially opened, the spigot drips water continually from a gravity feed into one end of the V-channel and keeps the dental roll wet enough to provide ample fresh water for the flies. The excess water drains into a gutter from the rear end of each trough. The dental rolls are replaced weekly or as they become soiled. Any deficiency in the water supply will reduce longevity of adults fed on a dry diet and may lower fecundity when a liquid diet is used.

Each cage is supplied with about 25,000 oriental fruit fly or melon fly pupae, 2 pounds of granulated or cubed sugar, and one-half pound of the yeast hydrolysate in rectangular paper plates. The Mediterranean fruit fly cages are stocked with 30,000 pupae, but because of their shorter egg-laying period these flies need only one-fourth as much sugar and protein as the other two species. By using strains that have been forced to adapt to a dry diet (separate sugar, yeast hydrolysate, and water), food is supplied only once. Average emergence for many millions of pupae of each species held under these conditions approximated 91, 93, and 87 percent for the oriental fruit fly, melon fly, and Mediterranean fruit fly, respectively, with the female-to-male ratio approximately 1:1 for all three species. Each cage generally contains from 11,000 to 13,000 pairs of flies at the start of production. Thus, the 8-cubic-foot cages allow a density of 3,000 or more flies

per cubic foot, which exceeds the density that could be maintained efficiently in the 11-inch cube cages previously used.

The collection of eggs from the Mediterranean and oriental fruit flies and the melon fly begins on the fourth, eighth, and ninth day, respectively, after emergence, although the Mediterranean fruit fly starts depositing fertile eggs on the third day and the other species on the seventh. The rich diet causes the flies to attain maximum fecundity in the second and third weeks. They usually produce considerably fewer eggs in the fourth or fifth week than they do on the average during the first 3 or 4 weeks, including the preovipositional period. This more rapid turnover in fly stocks, therefore, permits rearing the insects with fewer facilities and less labor for servicing them than when less efficient diets and lower temperatures that induced slower rates of development were used.

When the stacks of four cages were spaced less than 2 feet apart, egg production in the bottom cages averaged 20 to 36 percent less than that in the top cages. When the stacks were spaced 3 feet apart and the lighting system was modified to place more light in the lower cages, egg production among the cages was equalized. The subdued daylight and more uniform artificial lighting in the room reduced the excitability of flies and kept them from damaging each other by piling up in corners of the cages. Such controlled light also gave them more opportunity to feed undisturbed.

Collecting and Manipulating the Eggs

When eggs are collected from melon flies and oriental fruit flies three or more times per week over a 3-week period, the yield is about 2-1/2 and 3-1/2 million eggs per cage, respectively, with 40 to 60 percent of the females surviving at the end of this period. The Mediterranean fruit fly produces more than 2 million eggs per cage, with 20 to 50 percent of the flies surviving the 3-week holding period when eggs are collected nine times.

In 1957 the egg-collection method, which was developed in the 1929 Mediterranean fruit fly eradication campaign in Florida, and used by Finney (1956), was greatly simplified by eliminating the fresh section of orange rind and using instead a plastic lemon-shaped receptacle and later a quart-sized polyethylene container, both adapted by Tanaka (1965). The lemon-shaped receptacle is still used in egg-production cages of the 11-inch cube type. However, the larger polyethylene containers are used exclusively in the 8-cubic-foot cages (fig. 3). These containers are 9 inches long and taper from 3-1/4 to 3 inches in diameter. They fit snugly into each of the five 3-3/16-inch holes in the side of the cage and must be closed with tight-fitting plastic covers to prevent desiccation of eggs. Eggs are deposited through 0.3-mm. holes punched through the sides of the plastic container (fig. 4). About 300 holes are needed.

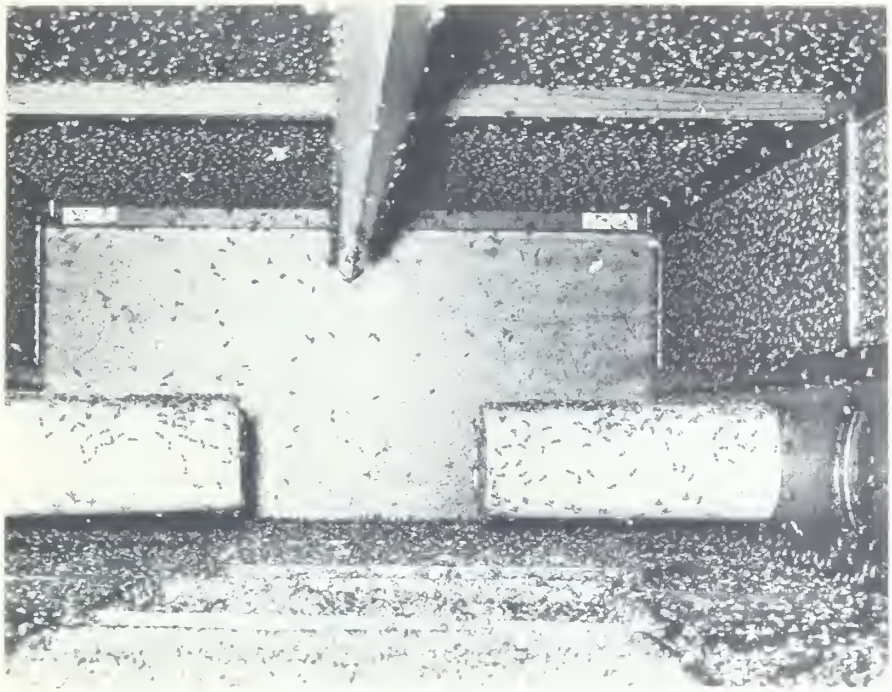

Figure 3.--Sugar, a special protein hydrolysate, and pupae are placed on the floor of the cage in separate paper plates. The food is introduced only once. A wick in the trough above provides ample fresh water. The perforated tubes are polyethyene egg-laying receptacles that fit through holes in the side of the cage.

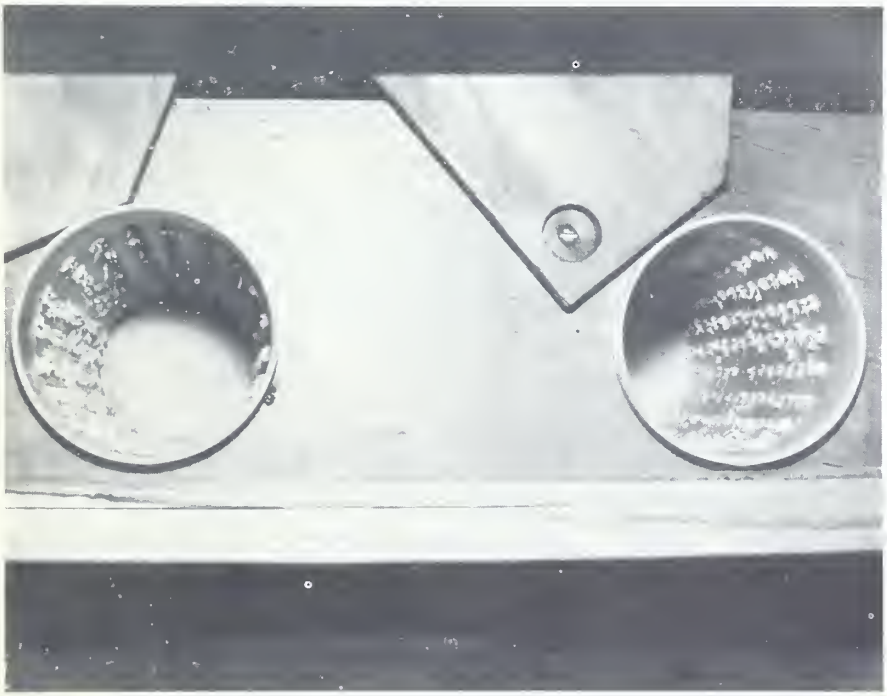

Figure 4.--The flies push their eggs through 0.3-mm. punctures. Before the receptacle is inserted, it is wet inside with warm water or fruit juice or equipped with a sponge wet with tomato juice, depending on the species. It also must be capped to prevent egg desiccation.

During the first 1 or 2 weeks of egg laying, six receptacles per cage give the best results. Larger numbers yield no more eggs. Later, as egg production diminishes, three or four are ample.

The inside of the egg receptacles (either the plastic lemon or the cylinder) used in the oriental fruit fly cages is coated with a solution of guava juice diluted with water. In the melon fly cages the egg receptacle is most effective when it contains a large cellulose sponge about 3 by 6 by 1 inches. The sponge, enclosed in a fine-mesh plastic screen to exclude eggs from the pores, is soaked in tomato juice diluted 1:1 with water before it is placed in the receptacle.

Until this procedure was developed at the laboratory, the melon fly could not be induced to deposit eggs in artificial receptacles. Formerly this species was produced by exposing cucumbers to ovipositing females and then, after hatch started, by transferring chunks of the cucumbers onto the larval medium. Because this procedure did not allow the laboratory workers to relate the known number of eggs or larvae to a given quantity of medium, the medium was sometimes overcrowded and at other times contained insufficient larvae to keep mold growth under control. Production was variable and unsatisfactory.

After the Mediterranean fruit fly became thoroughly adapted to the cages, the inside of the egg receptacle was coated with warm water. However, in the first 2 years of use, egg production of this fly was improved by coating the interior of the receptacle with lemon juice.

In the melon fly cages, when the flies had a choice, green receptacles induced a 50-percent greater egg deposition than yellow ones. When only one color was available, yellow was as effective as green. Neither the oriental fruit fly nor the Mediterranean fruit fly showed any color preference (Tanaka 1965).

During the usual daily work schedule from 8 to 4:30 the egg receptacles were placed in the cages before noon and removed the following morning. If needed, closer control of the age of the eggs can be obtained by utilizing shorter egg-laying periods. In the overnight periods, usually more than 80 percent of the eggs are deposited before dusk of the first day. After removal, the egg receptacles are placed in a container of water and the eggs washed out into a fine-mesh cloth bag. From the bag the eggs are transferred into a beaker, where they are kept under water for not more than 2 hours while they are divided into lots of about 40,000 eggs each. Oriental fruit fly eggs can be prevented from hatching by holding them in water at 80° F. with little retardation in their development. However, an adverse latent effect results when larvae from oriental fruit fly eggs are held in this manner. This method should be investigated further.

The eggs are measured volumetrically in a device consisting of a calibrated 3-inch section of a 5-ml. serological pipet having one end covered with organdy material and the other attached to the stem end (1/2 inch long) of a funnel held together with a small section of rubber tubing (fig. 5). First, the eggs are transferred to the desired volume in the pipet with a hypodermic needle and syringe having a 3-ml. capacity. Excess water drains out below

Figure 5.--Eggs are measured in water in units of approximately 40,000 or 50,000, then transferred to a cloth disk and held in plastic incubation dishes until hatching starts.

through the organdy. Lots of 40,000 eggs are transferred onto a moist cloth disk and held in a plastic lid 3 inches in diameter with projections around its rim to prevent sealing when the lids are stacked on top of each other. So as to keep the eggs moist until hatching begins, a thin film of water is placed under each disk.

Eggs of the oriental and Mediterranean fruit flies and the melon fly, held at 27° C., hatch in about 32, 44, and 23 hours, respectively (Messenger and Flitters 1958). In the Honolulu laboratory the melon fly eggs are placed on the larval medium the day they are collected and measured. The eggs of the oriental and Mediterranean fruit flies are incubated at 20° and 27°, respectively, overnight and are set on the medium 1 day after collection. This procedure permits them to be placed on the uncovered, very moist medium at the beginning of egg hatch. No protection against desiccation of eggs is necessary. Too late or too early setting, however, increases larval mortality. When variability in age of eggs is reduced by more frequent and shorter egg-laying periods and setting is accurately timed, a much higher percentage of the eggs will produce larvae that survive. Records of egg hatch are kept by withdrawing samples of eggs from each day's collections so that subsequently corrections can be made in numbers of eggs set if upward or downward trends in hatchability become evident.

Producing Larvae

For several years the fortified carrot medium of Finney (1956), with very little modification, has been used to produce oriental fruit flies in the Fruit Fly Investigations laboratory. This medium included pulverized raw carrots blended with 0.13 percent of Butoben,6/ 2 percent (by weight) of powdered brewers' yeast, and enough hydrochloric acid (HCl) to lower the pH from about 7 to 4.5. The Butoben, a mold inhibitor, was necessary to suppress the growth of undesirable molds and yeast. The brewers' yeast appeared to satisfy the absent or deficient dietary requirements in the carrots. The acid inhibited vigorous fermentation by lowering the pH.

6/ n-Butyl p-hydroxybenzoate (Merck).

Since 1953, 0.1 to 0.15 percent of sodium benzoate, instead of Butoben, has been used as the micro-organism inhibitor. In 1955 a dehydrated carrot powder (Christenson et al. 1956) was substituted for the pulverized fresh carrots. The dehydrated carrot powder greatly simplified preparation of the oriental fruit fly larval medium. In 1956 and 1957 a dehydrated carrot powder medium fortified with 2 percent of brewers' yeast powder and fresh papaya was used in producing Mediterranean fruit flies. Larval recovery from hatchable eggs on this and other formulas of medium ranged only from 16 to 43 percent during the period from 1952 to 1957. In 1958, by increasing the brewers' yeast to about 3 percent and omitting fresh papaya from the 1956 Mediterranean fruit fly medium, the average recovery rate was raised to 59 percent. The highest recovery rate with the melon fly medium was obtained when the brewers' yeast was increased to about 4 percent by weight. However, since increases in brewers' yeast make the medium more susceptible to mold growth, it must be carefully inspected from time to time. Currently, pupal production for each species is about 65 percent of the total number of eggs set.

The melon fly medium is slightly different from that of the other species. At first a more costly squash powder was substituted for the entire amount of dehydrated carrot powder. Later it was found that two-thirds of the squash powder could be replaced with carrot powder without any deleterious effect and with a substantial improvement in consistency of the medium and its performance.

The formulas now used at the laboratory in preparing the media for the three species are given in table 1.

Table 1.--Formulas used in preparation of media for Mediterranean and oriental fruit flies and melon flies

Material	Quantity (grams) per 100 liters for--	
	Mediterranean and oriental fruit flies	Melon fly
Sodium benzoate	100	100
Concentrated HCl	500 (420 ml.)	500 (420 ml.)
Brewers' yeast (powder)	3,200	4,250
Dehydrated squash (powder)	0	4,000
Dehydrated carrot (powder)	12,000	6,400
Water	1/ 90.7	1/ 91.3
Total weight (kg.)	106.5	106.5

1/ Liters.

These formulas require some adjustments in amount of acid to provide a pH of about 4.5 and also in amount of carrot powder or water to thicken or thin the mix. Differences in particles size and moisture content of the dehydrated vegetable powders affect the water-powder ratio. In addition, hand-mixed medium will not attain its thickest consistency in less than 2 hours, whereas continuous machine mixing will thicken the same formula in about 20 minutes. The difference in the way the formula is mixed must be considered in adjusting the formula and determining the best time for application of eggs or larvae.

Samples of dehydrated carrot and the squash powders should be tested to determine suitability and freedom from toxic residues. The purchase descriptions for each of these powders[7] are as follows:

Carrot powder:

(1) The carrot powder shall be made from mature, topped, Imperator or Red Cored Chantenay carrots, thoroughly washed, peeled with lye or steam, and washed again. The top 1-1/2 inches of the carrot root shall be removed and defective carrots or parts of carrots removed.

(2) The carrots shall be diced or sliced, washed, and blanched with steam to give a negative test for catalase. They shall then be dipped in a 2-percent food-grade starch solution to retard color and quality losses in the finished product.

(3) The dried carrots shall be ground into a powder just prior to shipment. The powder shall pass at least 98 percent through a U.S. Standard No. 60 (250 μ)sieve.

(4) The finished carrot powder shall contain not more than 4 percent of moisture [A.O.A.C. method 20.8 (1955)].

(5) The carrot powder shall have approximately the following percent composition: Protein 10, fat 4, fiber 9, ash 6, calcium 0.5, phosphorus 0.4, carotene 0.4-1, and carbohydrate 65.

(6) The finished product shall be packaged in a nitrogen atmosphere in plastic-lined cans or metal drums. The containers shall be marked with the date of grinding.

Squash powder:

(1) The squash powder shall be prepared from squash varieties that are clean, sound, matured, golden fleshed, firm shelled, and sweet. The fresh product shall be trimmed and all defects such as decay spots, sunburn, insect injury, and internal discoloration shall be removed.

7/ Prepared by chemists of the Entomology Research Division at Beltsville, Md.

(2) The fresh product shall be dried and ground in accordance with the standards of best commercial practice.

(3) The finished squash powder shall pass at least 98 percent through a U.S. Standard No. 60 (250 μ) sieve.

(4) The finished squash powder shall contain not more than 5 percent of moisture [A.O.A.C. method 20.8 (1955)].

(5) The finished squash powder shall be free from scorched or offcolor particles and should comply in all respects with the requirements of the Federal Food, Drug, and Cosmetic Act and General Regulations for its enforcement.

(6) The finished product shall be packaged in plastic-lined cans or metal drums. The containers shall be marked with the date of grinding.

At the Fruit Fly Investigations laboratory each medium prepared for large-scale production is mixed in batches of 180 liters in a 50-gallon vat with an electric mixer (fig. 6). Because of the hygroscopic nature of the

Figure 6.--A 50-gallon vat with an electric mixer facilitates the mixing of 180 liters of medium per batch to a consistency that is barely flowable. The medium consists of dehydrated carrot powder (alone or with squash powder), brewers' yeast, sodium benzoate, hydrochloric acid, and water.

carrot powder, the media are mixed in a room outside the air-conditioned facility so that dusts can be vented to the outdoors. First the vat is filled with the approximate amount of water required and then the sodium benzoate is added and allowed to mix for about 2 minutes before the acid is introduced. Brewers' yeast and dehydrated powder are added last. After thorough mixing, the acidity is adjusted by using more HCl, if necessary. Six liters of the mixture are drained into each of 30 aluminum trays lined with polyethylene plastic sheets (fig. 7). These trays are about 12 by 32 by 1-1/2 inches. They are filled with medium to a depth of about 1 inch. The plastic protects the tray from pitting by the acid medium.

Figure 7.--Before the medium thickens it is drained off into 6-liter trays lined with plastic sheets.

Two toilet paper strips, 27 by 2 inches wide, are placed across the top of each tray in contact with the medium. The cloth disk, containing about 40,000 oriental fruit or melon fly hatching eggs or 50,000 Mediterranean fruit fly eggs, is removed from the moist plastic chamber. The eggs and larvae are spread over eight or more small areas on each strip of toilet paper by touching the cloth held upside down to the strip (fig. 8). The strips disintegrate and disappear in the medium before the larvae reach maturity. Use of the toilet paper strips increases recovery of each species of flies by about 10 percent without adversely affecting their size. The strips also provide the larvae with an opportunity to hatch and disperse in the medium without immediate competition from larvae that hatch in aggregations of eggs.

Figure 8.--Hatching eggs are transferred to the medium by touching the cloth disks to single-ply toilet paper strips, which help larvae disperse and enter the medium.

After the eggs are distributed, the 30 trays of infested medium are placed in a double cabinet, 6 by 3 by 3 feet, having a screened center partition and 32- by 32-mesh plastic screen doors on each of its two sides (fig. 9). The

Figure 9.--Media cabinets mounted on casters have removable screened fronts and hold 15 of the 6-liter trays on each side of a screened partition. Large trays in the base catch mature larvae when they leave the media trays. From 0.7 to 0.9 million larvae per week can be produced in each cabinet.

cabinet, on casters, permits efficient positioning. The trays when in position are separated from each other by dowel supports. A deeper tray, 17 by 33 by 5 inches, half filled with fine vermiculite, is placed at the bottom on each side of the cabinet. Then the removable cabinet doors are sealed in place with masking tape to prevent entrance of Drosophila sp., which could breed more rapidly than fruit flies in the medium.

Mediterranean and oriental fruit fly larvae mature in 6 days and melon fly larvae in 5 days at 27° C. At the end of the larval period, the seal to the door is broken and the larvae that left the medium before 8 a.m. that day and dropped to the bottom tray are distributed with the vermiculite among pupation boxes. The remaining larvae (usually from 50 to 90 percent of the total) still in the medium are removed by slipping the plastic sheet with medium into the vat (fig. 10), diluting the larvae and medium residue with water, and

Figure 10.--After some mature larvae drop to the bottom tray, the others in the medium, along with the plastic sheet, are put into the vat and mixed with water.

after removal of the plastic, draining the slurry through a sieve. Each sieveful of larvae with adhering medium is washed under the tap (fig. 11), and the clean larvae are placed in a bucket of water until all 15 trays on one side of the cabinet have been washed (requiring about 15 minutes). The larvae recovered from 15 trays (usually 300,000 or more) are poured into a cloth bag to drain off excess water and are then transferred into a cement mixer, 4-1/2 cubic feet in capacity, containing 75 liters of vermiculite to which about 5 percent of water has been added. The larvae and pupation medium become well blended in the mixer, which is electrically driven at 25 r.p.m. for about 5 minutes. The resultant mix is transferred into a 5-cubic-foot box with

Figure 11.--The old media and larvae are drained from the vat and the larvae screened out and washed free of residue.

sloping sides and a bottom outlet. This box is raised on a hoist and from it the mix is discharged into each of 12 stackable, plastic boxes, 6 by 12 by 18 inches. Thus, about 1 liter of larvae (25,000 to 40,000 depending on the species) and 5 liters of vermiculite are placed in each box (fig. 12). The same procedure is used for the 15 trays on the other side of the cabinet.

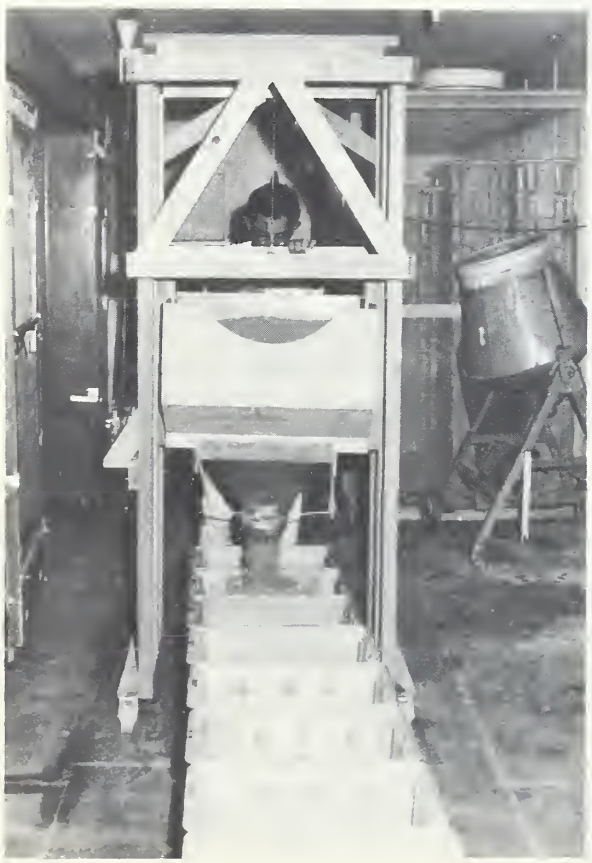

Figure 12.--After the washed mature larvae are blended with vermiculite in a cement mixer, the resultant mix is distributed among plastic stackable boxes, where it is held while the larvae pupate.

The larval culture trays and cabinets are then flushed out and steam sterilized to prepare them for reuse. In addition to sterilizing the equipment, the steam kills any eggs, larvae, or pupae of Drosophila sp. that may be in the cabinet as the result of penetration of adult flies. Infestations of Drosophila sp. often seriously affected production before steam-sterilizing was used.

By using these cabinets with 30 trays each, from 700,000 to 900,000 mature larvae can be produced in 1 week or less. These represent average yields of about 25 to 32 pupae per gram of nonwater media ingredients. Each of the 30 trays is set with 40,000 oriental fruit fly or melon fly eggs, or about 50,000 Mediterranean fruit fly eggs. It is essential that a high hatch rate be maintained and that eggs be of the proper age to provide the maximum number of larvae in the correct stage at time of application or soon thereafter. During the past 2 years pupal production rates have averaged about 65 percent of the total number of eggs set, even though more larvae have been used for a given amount of medium than in previous years.

When the three species were produced in thick media that became moderately dry, high larval concentrations caused temperature increases to 35.5° C., or 8° above the ambient in the media during the 24-hour period immediately before larval maturity. The temperature fell to the ambient level as larvae vacated the media.

Processing Pupae

Pupae must be handled carefully to avoid damage from desiccation, overheating, or concussion. For many years screened, washed sand was used as a pupation medium. But sometimes during and after pupation as many as 40 percent of the insects died, particularly if the sand was excessively moist or was overloaded with larvae. Because of the weight of the sand, pupae were also subject to some mechanical damage when they were sifted out.

After testing several products and mixtures, a nearly dust-free grade of vermiculite, designated as Finish Plaster 4, was adopted. The insects can tolerate a moisture content ranging from 3 to 10 percent by weight, but they withstand excess amounts of moisture in the vermiculite far better than similar amounts in sand. While larvae are pupating, a moisture content of 3 to 5 percent is required in either sand or vermiculite. It is unnecessary to add additional water. Vermiculite induces the larvae to stay or crawl below the surface and pupate rapidly, generally within the first 24-hour period. Sand that is too moist repels larvae so that they attempt to crawl out of the containers and thus pupation is delayed.

After pupae are mixed in the vermiculite, they should be left undisturbed for at least 2 days at 27° C. to avoid damaging them. The same vermiculite has been used repeatedly without sterilization in the Fruit Fly Investigations laboratory, although sterilization might become necessary if disease appears. Exhaust fans and face masks are used in this laboratory as safety measures to avoid inhaling vermiculite dust.

In order to obtain maximum efficiency in the production facility, it is essential to maintain almost continuous production. However, pupae for large-scale releases were needed only once or twice a week. Therefore, it was necessary to hold each lot of pupae under different temperature conditions so that development would proceed at rates that would bring the different lots to the same stage 48 hours before emergence. At this time they could be pooled and shipped or held for simultaneous emergence.

At 27° C. the average pupal period for the oriental and Mediterranean fruit flies was 10 days and that for the melon fly 9 days. By comparing the effects of a range of constant temperatures from 10° to 30° on the rate of pupal development of each species, it was found that 20° slowed development to half the rate noted at 27°. Since all mature larvae were held for 2 days at 27°, or until after pupation was completed, the time of development was adjusted by varying the time that they were held at 27° and at 20°. The pupae were left in the vermiculite until placed at 20°. For example, six lots of larvae that pupated on successive days were programed for incubation at 27° for 2, 3, 4, 5, 6, and 7 days, but at 20° for 10, 8, 6, 4, 2, and 0 days, respectively. Larvae then would all be at the same stage of development by the end of the period. Those pupae that could not be developed fast enough to meet a deadline were slowed in their development to meet the following week's requirements. Most flies so held emerged within a 40-hour period starting 1 day after the blending of lots.

With a mechanical sifter devised in 1962, the time required to separate 1 million pupae from the vermiculite was reduced from 12 man-hours to about one-half man-hour. The sifting device consists of two trays with 12-mesh screen bottoms that move on parallel tracks with 1-inch horizontal strokes controlled by a motor-driven eccentric (fig. 13). By operating this device for

Figure 13.--Pupae are separated from the vermiculite by a mechanical shaker at a rate of more than 2 million per hour.

5 seconds at 160 strokes per minute, the pupae can be completely separated from the vermiculite. At this speed pupae can be left on the sifter for up to 15 seconds without damage. However, at speeds of 240 strokes per minute, shaking on the sifter for 10 and 15 seconds has caused pupal mortality of 15 and 65 percent, respectively. Older pupae are somewhat more tolerant than pupae under 5 days of age.

Melon fly emergence from 73 million pupae, which were formed in the sand during 1959-61, averaged 81 percent. In the 3 years since vermiculite was substituted, emergence from 250 million pupae has averaged 93 percent.

After sifting, the pupae are spread about 1 cm. deep in a screen-bottomed tray, 20 by 20 by 1 inches (inside dimensions), and transferred to a room held at 20° C., where the relative humidity is above 85 percent to prevent damage by desiccation (fig. 14). Some pupae must be held in a naked condition in this room for more than a week.

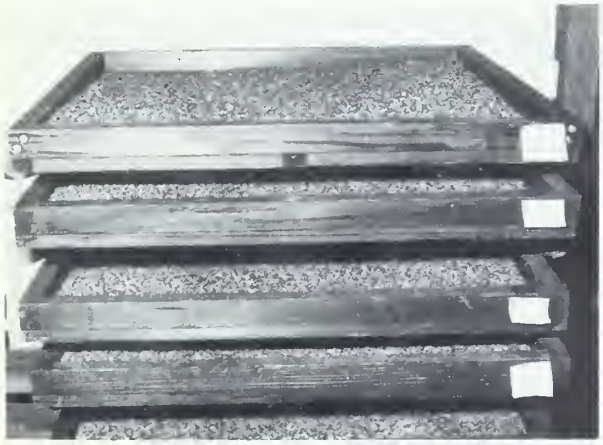

Figure 14.--Naked pupae are subject to overheating and desiccation. To avoid such damage, they are held at 20° C. and 85- to 90-percent relative humidity in shallow screen-bottomed trays until ready for use. An oscillating fan helps maintain a uniform temperature.

Trays are separated by 1-inch cleats. An oscillating fan, beginning at least 3 days before the expected emergence date, is used to prevent heat accumulation between and in trays. The shallow depth and screen-bottomed trays help prevent overheating in the maturing pupae. In poorly ventilated trays or when depths greater than 1 cm. are used, temperatures will usually increase rapidly to lethal levels. Those pupae that emerge after having been overheated produce weaker, shorter lived flies.

Production Costs

Costs per million pupae for fly food, wicks, larval-medium components, and other expendable items approximate $41 for the oriental or Mediterranean fruit flies and $46 for the melon fly. These costs are based on cage yields of 2 million eggs per generation and on pupal recovery rates averaging 65 percent of the eggs set.

Labor requirements with present facilities and highly skilled workers approximate 5-1/2 man-hours per million pupae. This time includes 15 minutes for steam-cleaning and washing cages, preparing food, and changing wicks; 40

minutes for all egg-production operations, including determination of average hatch rate; 80 minutes for preparing and setting medium; 115 minutes for collecting or washing larvae from medium, mixing them with vermiculite, placing in boxes, and cleaning plastic sheets; 35 minutes for steam-cleaning medium cabinets and trays; 25 minutes for sifting pupae from vermiculite, distributing them in holding trays, and determining percent emergence; and 20 minutes for miscellaneous items, such as cutting wicks, tissue, plastic sheets, and egg disks.

Discussion

The production methods discussed in this report have been continuously modified. The changes necessary to accommodate any one of the three species are simple. However, further cost reductions could be made if production could be limited to only one species of fly.

In the preliminary stages of production many formulations of media were tested as replacements for the dehydrated carrot powder, sodium benzoate, and brewers' yeast. These included dehydrated bananas, potatoes, and plankton, different antibiotics and micro-organism inhibitors, and sources of protein other than brewers' yeast. The media, as developed, appear to be the best available for the particular methods of handling. Modifications in the formula might require use of other methods of mixing, setting, and holding of media. A slightly thicker medium would permit greater production of full-sized larvae per unit of space by the addition of more eggs to the medium, but might also require more labor and time for preparation and distribution. Egg production per cage can be increased by daily instead of less frequent egg collecting.

The maintenance of careful, regular quality control tests is important. In these tests samples must be taken and determinations made as to percentages of egg hatch, larval returns from eggs, numbers of mature larvae that pupate successfully, pupal size, and adult emergence. Less frequently adult compatibility with wild flies and longevity of reared flies released into a natural environment must also be tested.

A low hatching rate, poor distribution of eggs or larvae on the medium, or an insufficient number of surviving larvae may also permit molds and fungi to develop. So as to prevent this, sodium benzoate can be used at concentrations up to 0.15 percent, if needed; but concentrations of 0.20 percent or more affect larval development adversely and reduce survival. It is important that the trays be loaded with the greatest number of larvae that the medium can support without greatly reducing pupal size.

Excess humidity in the egg-production facility makes it impossible to obtain effective results with a dry adult diet. When both high temperatures and high humidity prevail in this room, the stock flies usually die rapidly. Although a moderate humidity is needed in the adult fly environment, the egg containers must be closed and their insides kept moist to prevent desiccation of eggs. Desiccation may also result if oviposition holes are too large or too numerous. Both eggs and newly hatched larvae must be protected from desiccation during the holding period, especially if placed on medium that does not have a film of moisture on the surface.

Pupae lose some weight throughout the pupal period. If held naked in an air-conditioned facility, where the relative humidity is not maintained above 85 percent, pupae will lose enough moisture to significantly reduce the percentage of their emergence. Most stress situations that increase mortality among pupae result in adverse latent effects on surviving flies, which generally do not live as long as flies reared under optimum conditions.

Summary

Improved or new adult insect diets, larval media, special methods, and equipment have been developed at the Fruit Fly Investigations laboratory in Honolulu for highly efficient laboratory production of the oriental fruit fly (Dacus dorsalis Hendel), the melon fly (D. cucurbitae Coquillett), and the Mediterranean fruit fly [Ceratitis capitata (Wiedemann)].

Under controlled temperature and partially regulated humidity and light conditions, cages stocked with 11,000 to 13,000 pairs of flies yield from 2 to 3-1/2 million eggs each during the 3- to 4-week holding period. Eggs are deposited in plastic receptacles inserted through the sides of the cage. Flies have constant access to a dry diet of yeast hydrolysate and sugar, with adequate fresh water available. Only one stocking of the cage with food is necessary. Pupal recoveries average 65 percent of the total eggs set on a simple uncooked medium made from water, dehydrated carrots (or squash), brewers' yeast powder, sodium benzoate, and sufficient hydrochloric acid to bring the pH to 4.5. The yield of pupae ranges from 25 to 32 per gram of nonwater media ingredients. Hatching eggs are placed on exposed nonsterile medium, larvae emerge or are washed out, pupation occurs in vermiculite, and the pupae are later separated by mechanical means. They are held at appropriate temperatures to regulate development before blending the different lots that emerge concurrently.

Production costs have been reduced to less than $46 per million pupae for all rearing supplies. Labor requirements now approximate 5-1/2 man-hours per million pupae. About 1.4 billion of the three species were produced from 1960 to 1964, inclusive, while production research was in progress.

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